Article

Quercetin and *trans*-resveratrol phytoestrogens assay in Morellino di Scansano wine samples

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Summary

Besides the basic nutritional compounds the human diet contains a series of natural essences, that without having specific nutritional characteristics show biological activity, and can therefore be referred as phytochemical compounds. The red wine is a discrete source of phytochemical compounds positively interacting with the human organism. Among them, quercetin together with *trans*-resveratrol are the most represented. In particular wine represents the main source of resveratrol, being fruits and vegetables the most relevant source of quercetin.
The aim of the study was to determine phytoestrogens quercetin and trans-resveratrol in samples of Tuscany red wine. In order to do this, 13 wine samples from 7 producers of the area of Morellino di Scansano (Grosseto, Italy) and 13 wine samples obtained from the Chianti area (Siena, Italy) were analysed and compared.
All the analysed samples were directly picked up from the bottle, protected from the sunlight and injected in HPLC system with diode array scan system, without any purification, extraction or filtration procedure of the wine samples.
The data we obtained showed that quercetin is highly present in all the samples, with a mean value almost always above 10 mg/L and with a peak of about 30 mg/L. Similarly, the results obtained in the *trans*-resveratrol assays showed that the values found in samples of the Morellino and Chianti wines were, on the average, slightly higher than those reported in the international literature.
In conclusion, from our analyses we observed that the phytoestrogens (quercetin and resveratrol) in the studied Tuscany red wines are well represented and correlate with grapevine variety and the soil.

KEY WORDS: phytoestrogens, wine, HPLC analysis.

Introduction

Besides the basic nutritional compounds, as macro and micro nourishments, the human diet contains a series of natural essences, that without having specific nutritional characteristics, show biological activity, and can therefore be referred as *phytochemical compounds* (1). In countries where wine is widely consumed, this beverage is a discrete source of polyphenolic compounds which belong to the above defined group of substances. Among them, quercetin and its glycosylated congeners (for example, rutin and quercitrin) together with cis- and trans-resveratrol (non-flavonoid polyphenols) and its glycosides are the most represented in red wine. The metabolism of this compounds take place during fermentation of grape, when the sugar moiety can be partially or totally separated and during digestion where both the gut microflora and the liver play an active role (2).
The polyphenolic content of wine depends on the grapevine variety, the type of soil, the climate, the method of wine production and conservation (3). Therefore every production, even if it occurs in the same geographical area, can have a significant impact on the quality and quantity of these substances.
The polyphenolic compounds in wine have been evaluated to ascertain their effects on human health. In particular, substances such as quercetin and trans-resveratrol have shown a potent anti-oxidative activity, even ten times greater than the analogous activity of tocopherols, and weak estrogenic activity (4, 5). An ample debate is also currently under way on their effectiveness as anti-mitogens in conditions such as colon and breast tumors. These questions are far from being answered, because of the lack of clear epidemiological analyses, but recent publications indicate a protective effect of quercetin and *trans*-resveratrol against these pathologies (6, 7). Wine represents the main source of these compounds, expecially referring to resveratrol, being fruits and vegetables a relevant source of quercetin.
With these premises we have evaluated the content of quercetin and trans-resveratrol in red wines obtained from two important production areas within the Tuscany Region.

Materials and methods

A wide sampling of typical Italian wines, commonly found in the market, originating from two different geographical areas of Tuscany: the Grossetano area of the Morellino di Scansano (tirteen different wines) and the Siena Chianti area (tirteen different wines), were evaluated for their content in quercetin and trans-resveratrol. The separation and determination of these flavonoids were obtained according to the method described by Frankie et al. (8), appropriately modified in the gradient timing.
Methanol, acetic acid (glacial) 100%, acetonitrile, dichloromethane, water and all solvents used for HPLC of analytical or HPLC grade were purchased from Merck (Darmstadt, Germany). Quercetin and trans-resveratrol used for the stock solutions were obtained from Extrasynthèse (Gigalabo) and from Sigma-Aldrich (Milwaukee, W, USA), respectively. Standard solutions used to obtain the calibration curve were prepared dissolving 5 mg of standard in 10 ml of ethanol. Ultra-pure water from a Milli-Q Millipore system (Bedford, MA, USA) was used whenever a washing was performed. All the eluents were sonicated for 5 minutes before the use.

The analyses were carried out on 20 µl samples from Morellino and Chianti wines (five samples from each wine were tested), at room temperature, with a dual pump 515 model (Waters, MA, USA) liquid chromatographic system, supplied with a diode array system Model 996 (Waters, MA, USA). A Waters Millennium 32 software was used. Quercetin was detected at 366 nm while the *trans*-resveratrol was observed at 306 nm (Figure 1). We used an HPLC analytical reversed-phase column Novapak C_{18} (Waters, MA, USA) 3.9 × 150 mm fitted with a pre-column Sentry Simmetry C_{18} (Waters, MA, USA). Elution was performed at a flow rate of 0.8 ml/min with the following linear gradient: A = water/acetic acid (9:1 v/v); B = methanol/acetonitrile/dichlorometane (10:5:1 v/v/v), B in A (v/v): 5% to 45% in 20 min and from 45% to 5% in 5 min with equilibration for 10 min before subsequent injection.
Data were statistically analyzed by STATISTICA 6.0 software.

Results and discussion

The epidemiological data available show that a moderate consumption of red wine produces in man very different effects from those produced by equivalent quantities of alcohol and partly different effects from those linked with the consumption of white wine (9). The protective action of cardiovascular disorders is the most relevant observation in conditions of moderate consumption of red wines (4). These benefits have been related to the protective effect against free radicals and linked with the content of phytoestrogenic molecules, as quercetin and *trans*-resveratrol (6, 7). The importance of these molecules and their identification as "phytoestrogens" is based on the structural and functional similarity with diethylstilbestrol, a bioactive synthetic estrogen. Resveratrol, usually present in detectable quantities in red wines, as the *trans* isomer, exhibits *in vitro* both anti-oxidizing activities, by inhibiting LDL oxidation, and estrogenic agonistic activities (10). It should be noted that the anti-oxidant action *versus* the intact LDL is performed by the red wine, while the ingestion of the berry *in toto* has a much less intense effect.

The Chianti area is famous throughout the world for its production of fine wines. Many studies have confirmed the high nutritional power of these wines and verified the presence of quercetin and *trans*-resveratrol (4, 11). Less renown are, instead, the characteristics of the wines from the Morellino di Scansano area for which no data are present in the scientific literature.
The data we have obtained showed that quercetin is highly present in all the samples, with values almost always above 10 mg/l with a peak of about 20 mg/l in one of the samples (Table I). The average of quercetin, expressed in mg/l, in the wines from the Grosseto area (13.36 ± 2.51 mg/l) is higher, even though not significantly, than that obtained for the wine from the Siena Chianti area (11.10 ± 3.27 mg/l). Evaluation of homologous wine series, respectively Morellino and Chianti, showed a significant difference among the various production companies ($p < 0.05$).
Compared with the data obtained by other authors concerning the wines of various origin and different years, where the average quercetin values range between 1 and 10 mg/l (11), the concentrations we found in the Morellino and Chianti wines were constantly higher, especially for the Morellino di Scansano bordolese wines. Similarly the results obtained in the trans-resveratrol assays showed that the values found in samples of the Morellino and Chianti wines were, on the average, slightly higher than those reported in the literature [average *trans*-resveratrol value: 1-2 mg/l (5)]. This is particularly true for the Chianti bordolese wines for which the average value is 2.80 ± 0.71 mg/l, while for the Morellino di Scansano wines the value found was 2.50 ± 1.22 mg/l. Furthermore, from our analyses resulted that the samples drawn and analyzed 24 hours after opening the bottles, showed the same unaltered values both for quercetin and for trans-resveratrol.
In conclusion from this analyses we have observed as the two phytochemicals analyzed here are well represented in Tuscany red wines. Future studies will attempt to answer the question related to the differences observed among the various geographical and production areas.

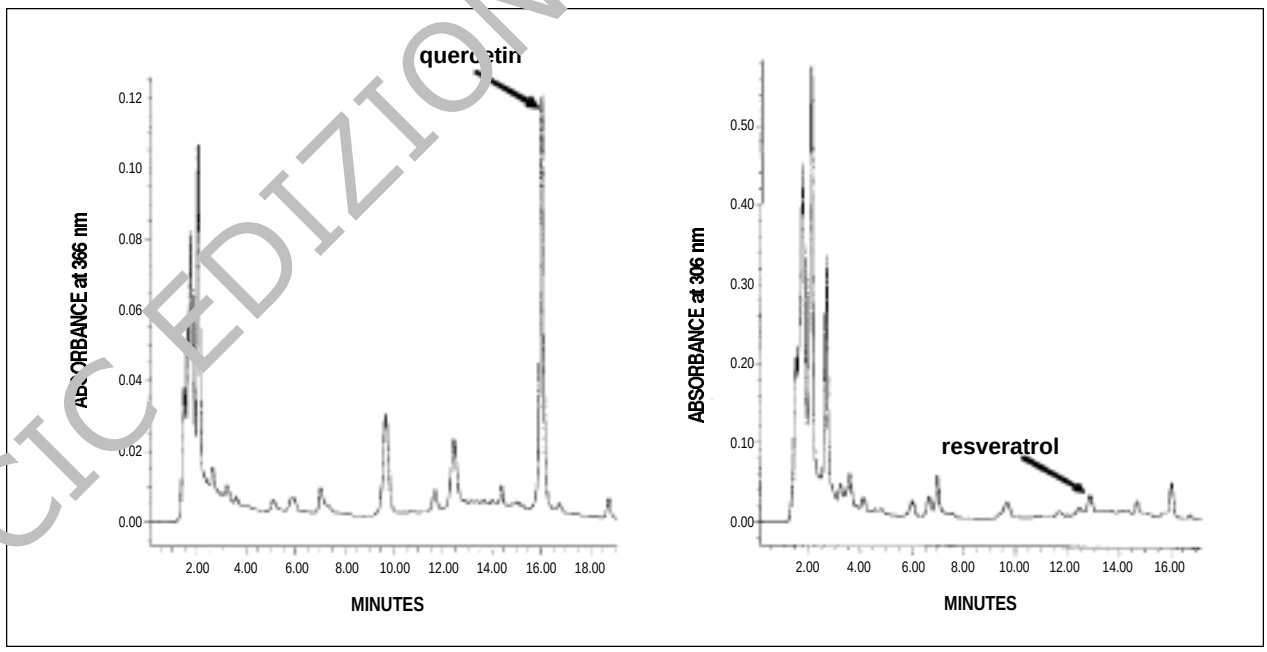

Figure 1 - HPLC elution of quercetin and resveratrol.

Table I - Quercetin and resveratrol quantities measured in samples of Morellino and Chianti wines, immediately after bottle opening. Each wine sample was tested 5 times, data were expressed as mean ± standard deviation.

	QUERCETIN		RESVERATROL	
	Mean[(a)]	Std. dev.[(b)]	Mean[(a)]	Std. dev.[(b)]
MORELLINO WINES				
1	9.86	0.05	2.26	0.01
2	16.02	0.16	1.96	0.02
3	10.64	0.99	3.82	0.03
4	13.45	0.65	3.69	0.07
5	14.02	0.12	3.14	0.07
6	19.43	0.60	2.58	0.37
7	14.33	0.64	1.96	0.06
8	11.74	0.10	2.55	0.13
9	11.56	0.46	3.78	0.35
10	14.18	0.52	2.92	0.10
11	12.13	0.26	3.54	0.07
12	12.00	0.13	3.54	0.07
13	14.39	0.17	0.19	0.07
MEAN	13.36	2.51	2.50	1.22
	QUERCETIN		RESVERATROL	
	Mean[(a)]	Std. dev.[(b)]	Mean[(a)]	Std. dev.[(b)]
CHIANTI WINES				
1	13.82	0.24	3.44	0.20
2	14.61	0.17	4.09	0.08
3	9.13	1.92	2.12	0.43
4	10.35	0.28	2.67	0.09
5	16.27	0.35	N.P.	0.13
6	12.70	0.10	2.12	0.07
7	10.01	0.12	2.09	0.08
8	8.93	0.29	2.34	0.14
9	11.64	0.22	2.07	0.09
10	5.78	0.06	3.27	0.10
11	10.33	0.26	3.64	0.0
12	14.85	0.44	3.43	0.08
13	5.97	0.49	2.50	0.1
MEAN	11.10	3.27	2.82	0.71

[(a)] In mg/l
[(b)] Computed with the software STATISTICA 6.0.

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